Analysis Results

Date received	December 10 2014	Client	CNL
Name of sample	Silver ion water		

Below are the results of efficacy evaluation of the compound provided by the client.

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■ Preventive effect on seven plant diseases by simultaneous treatment of silver ion water and pathogen

1. Test methods

Subject diseases: Plant diseases used for the experiment to validate *in vivo* disinfection activity of silver ion water are as follows:

Table 1. Plant diseases subject to the validation of preventive effects against plant diseases

Plant disease (Korean)	Plant disease (English)	Pathogen	Abbreviation
벼 도열병	Rice blast	*Magnaporthe oryzae*	RCB
벼 잎집무늬마름병	Rice sheath blight	*Rhizoctonia solani*	RSB
토마토 잿빛곰팡이병	Tomato gray mold	*Botrytis cinerea*	TGM
토마토 역병	Tomato late blight	*Phytophthora infestants*	TLB
밀 붉은녹병	Wheat leaf rust	*Puccinia recondite*	WLR
보리 흰가루병	Barley powdery mildew	*Blumeria graminis f. sp. hordei*	BPM
고추 탄저병	Pepper anthracnose	*Collectotrichum coccodes*	PAN

Preparation and treatment of drug: Tween 20 as surfactant was added to 1, 2, 4, and 10 ppm silver ion water produced by the client's silver ion water maker respectively to finally reach a concentration of 250 ppm in the drug solutions. The same amount of pathogens as described below was mixed with the solutions and the solutions were applied evenly throughout the plants. Two pots of seedlings were used per treatment for each subject disease.

Preparation of pathogens and investigation of plant disease:

For RCB, *Magnaporthe oryzae* KI-1113a strain was inoculated to a rice polish agar medium (rice polish 20g, dextrose 10g, agar 15g, and distilled water 1 l) and cultured for two weeks at 25℃. The surface of the cultured medium was scraped by a rubber polishman to remove submerged hyphae and spores were formed on a shelf with fluorescent lighting (25 - 28℃) for 48 hours. For the inoculation of RCB, a spore suspension at a certain concentration (10 x 10^6 conidia/ml) was made with conidiospores and sterilized distilled water, mixed with the same amount of drugs, and sprayed over Chucheong rice plant (with 2 – 3 foliage leaves). The inoculated rice plant was left in a dark wet room for 24 hours and in a constant temperature and humidity room at 26℃ with a relative humidity of at least 80% for five days to bring to disease outbreak. Then diseased rate (%) was investigated.

For RSB, a moderate amount of wheat bran was sterilized in a 1L culture bottle. *Rhizoctonia solani* AG-1 strain was inoculated to a sterilized medium and cultured for seven days at 25℃. The cultured mycelium lumps were finely ground, mixed with the same amount of drugs, and inoculated by pouring over the pot of Chucheong rice plants with 3 – 4 leaves, which were brought to disease outbreak by culturing in a wet room (25℃) for seven days. The outbreak of the disease was observed by investigating diseased rate of the leaf sheath.

For TGM, *Botrytis cinerea* was inoculated to a potato agar medium and cultured for seven days in a constant temperature device (dark conditions) at 25℃, followed by culture for seven days with 12 hours of light exposure per day to form spores. For the inoculation of TGM, the cultured spores were harvested with a potato dextrose broth. The concentration of the spores was set to 5 x 10^5 conidia/ml with a hemocytometer and the spores were mixed with the same amount of drugs and inoculated by spraying over tomato seedlings (with 2 – 3 foliage leaves). The inoculated tomato seedlings were brought to disease outbreak by leaving in a wet room (with a relative humidity over 95%) at 20℃ for three days. Diseased rate (%) was observed on the leaves.

For TLB, *Phytophthora infestants* PIT strain was inoculated to an oatmeal agar medium and cultured for seven days in a constant temperature device (dark conditions) at 20℃, followed by culture for seven days with 16 hours of light exposure per day to form zoosporangium. The zoosporangium were harvested by adding sterilized distilled water and the concentration of spores was investigated with a hemocytometer under an optical microscope to make a spore suspension of 5 x 10^5 sporangia/ml, which was cold-processed for 1 hour at 4℃ to bring out zoospores, mixed with the same amount of drugs, and sprayed over tomato seedlings (with 2 – 3 foliage leaves). The inoculated tomato seedlings were left in a wet room at 20℃ for two days and then moved to a constant temperature room at 20℃, and diseased rate (%) was investigated.

For WLR, given the pathogen *Puccinia recondite* is a parasite, it was subcultured directly on plants in the laboratory, and spores formed on wheat seedlings were used as the source of inoculation. To investigate to efficacy of the drugs, five wheat seeds (‘Eunpa’) were planted per pot (diameter: 6.5 cm) and cultured for eight days in a greenhouse, and a spore suspension (spores 0.67g/L) was mixed with the same amount of drugs and sprayed over wheat seedlings with one leaf. The inoculated wheat seedlings were left in a wet room at 20℃ for one day and moved to a constant temperature and humidity room at 20℃ with a relative temperature of 60% to bring to disease outbreak. Diseased rate was investigated seven days after inoculation.

For BPM, given the pathogen *Blumeria graminis f. sp. horder* is a parasite, it was subcultured directly on barley seedlings in the laboratory, and spores formed on the barley seedlings were used as the source of inoculation. To investigate to efficacy of the drugs, five barley seeds (‘Dongbori’) were planted per pot (diameter: 6.5 cm) and cultured for eight days in a greenhouse. The cultured one-leaf barley seedlings were treated with silver ion water and BPM spores were dusted over the barley seedlings. The inoculated barley seedlings were left in a constant temperature and humidity room at 20℃ with a relative temperature of 60% for seven days to bring to disease outbreak, and diseased rate was investigated.

Calculation of control rate: Based on the diseased rates obtained from the experiments, control rate was calculated by the following formula:

Control rate (%) = (1 – diseased rate of treated group / diseased rate of untreated group) x 100.

2. Results

Table 2. Preventive effects on seven plant diseases by simultaneous ion water treatment

Treatment concentration (ppm)	RCB[a]	RSB	TGM	TLB	WLR	BPM	PAN
0.5	83	15	50	96	0	0	0
1	100	20	50	96	3	0	38
2	100	15	50	98	0	0	25
5	100	15	50	99	0	8	13

a RCB, Rice blast; RSB, Rice sheath blight; TGM, Tomato gray mold; TLB, Tomato late blight; WLR, Wheat leaf rust; BPM, Barley powdery mildew; and PAN, Pepper anthracnose.
b Control rate (%).

Table 3. Plant disease preventive effects of single treatment of control drugs one day prior to inoculation

Chemical	Con (μg, ml)	RCB[a]	RSB	TGM	TLB	WLR	BPM	PAN
Blasticidic-S	50	100[b]						
	1	83						
Tricyclazole	10	100						
	0.5	95						
Validamycin	50		100					
	5		90					
Flutolanil	50		100					
	20		100					
Fludioxonil	50			100				
	5			82				
Fenheximide	100			100				
	20			82				
Dimenthomorph	10				100			
	2				97			
Chlorothalonil	100				100			
	50				100			
Flusilazole	10					100		
	2					60		
Carboxin	50					100		
	20					67		
Flusilazole	10						100	
	0.5						90	
Benomyl	100						100	
	1						92	
Dithianon	50							88
	10							55

a RCB, Rice blast; RSB, Rice sheath blight; TGM, Tomato gray mold; TLB, Tomato late blight; WLR, Wheat leaf rust; BPM, Barley powdery mildew; and PAN, Pepper anthracnose.
b Control rate (%).